DATA EVALUATION RECORD
HONEY BEE - FIELD TESTING FOR POLLINATORS, §141-5

1. **CHEMICAL:** Novaluron — PC Code No.: 124002

2. **TEST MATERIAL:** "RIMON" 10EC — Purity: 9.1%

3. **CITATION:**

Author:	Gray, A.P.
Title:	"RIMON" 10EC, Evaluation of the Effects of Insect Growth-Regulating Insecticides on Honey Bee (*Apis mellifera*) Colony Brood Development.
Study Completion Date:	June 12, 1998
Laboratory:	Huntingdon Life Sciences Ltd. Huntingdon , Cambridgeshire, England
Sponsor:	Makhteshim Chemical Works Ltd. Beer-Sheva, Israel
Laboratory Report ID:	MAK 435/973449
DP Barcode:	D285479
MRID No.:	45638407

4. **REVIEWED BY:** Rebecca Bryan, Staff Scientist, Dynamac Corporation.

Signature: Rebecca Bryan **Date:** 4/1/03

APPROVED BY: Teri Myers, Ph.D., Staff Scientist, Dynamac Corporation

Signature: [signature] **Date:** 4/1/03

5. **APPROVED BY:** Bill Evans, ERB I

Signature: [signature] **Date:** 11/21/04

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6. **STUDY PARAMETERS:**

Scientific Name of Test Organism: *Apis mellifera*
Definitive Study Duration: July 22-August 12, 1997 (21 days)

7. **CONCLUSIONS:** This study examined the effect of "RIMON" 10EC on honey bee colony brood development. Four beehives (about 10,000 to 15,000 bees each) per treatment and control were placed in a grass field and bees were fed 50% w/v sucrose solutions using commercial hive feeders. The negative control bees were fed an untreated sucrose solution and the treatment group bees were fed sucrose solution containing a nominal concentration of 3.3 mL "RIMON" 10EC/L. A reference toxicant, diflubenzuron 480 g/L SC, was tested at a nominal concentration of 1.2 mL of product/L.

There were significant adverse effects ($p<0.05$) on brood development at all stages (i.e., eggs, young larvae, and old larvae) in the 3.3 mL"RIMON" 10EC/L treatment group compared to the control, with reductions in successful development appearing in young larvae 2 days after treatment and in eggs and old larvae 7 days after treatment; these adverse effects on development persisted over the 21-day study. There was no significant difference between mortality of the sucrose control and "RIMON" 10EC treatment group for any developmental stage (i.e., workers, drones, pupae, and larvae). No other treatment related effects were observed during the study. The LC_{50} could not be determined, but it was presumed to be >3.3 mL"RIMON" 10EC/L.

The study is classified as Supplemental because there is not an EPA-approved protocol for this type of study. The study is scientifically sound and the information that it provides may be useful for risk assessment purposes.

8. **ADEQUACY OF THE STUDY:**

A. Classification: Supplemental

B. Rationale: These studies are only required on a case-by-case basis. A protocol was not approved by the EPA for this insect field study, but it provides useful information for risk assessment purposes.

C. Repairability: None

9. GUIDELINE DEVIATIONS: N/A

10. SUBMISSION PURPOSE: This study was submitted to evaluate the effect of "RIMON" 10EC on the honey bee colony brood development.

11. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
Species: Species of concern (*Apis mellifera*, *Megachile rotundata*, or *Nomia melanderi*)	*Apis mellifera*
Age at beginning of test:	National and commercial type colonies with all life-stages present. Hives were set up 4-7 days before dose application.
Pre-test health:	Hives were healthy, well fed, and queen-right colonies.
Supplier	Mr. R. Baker, 19 Abbotts Crescent, St. Ives, Cambridgeshire, UK.
All bees from the same source?	Yes

B. Test System

Guideline Criteria	Reported Information
Site Characterization:	• A grass field located within the perimeter fence at Huntingdon Life Sciences. • The test field had few potential sources of nectar or pollen. • There were potential sources of food within 0.5 miles (south western edge of perimeter fence) and 2.0 km (towns of Alconury and Woolley), (p. 15). • Temperature (air and soil), relative humidity, and precipitation events were recorded hourly using a portable weather station.
Trap descriptions:	Dead bee traps were black plastic trays (50 x 50 x 30 cm) with a wire mesh top (1.5 cm mesh). The traps were placed in front of the bee hives, so bees exiting the hive were required to pass through the wire mesh.
Number of Plots/Treatment:	Four replicate hive colonies per treatment and control group. Brood development for each replicate was determined from the 100 cells with eggs, 100 cells with young larvae, and 100 cells with old larvae designated 33 hours prior to treatment.
Food Preparation:	The bees were allowed to obtain food by foraging natural surrounding nectar sources. No supplementary food was provided.

Guideline Criteria	Reported Information
Precipitation:	0-1.4 mm (mean of 0.03 mm)
Temperature:	Air: 8.78-31.07°C (mean of 19.25°C) Soil: 15.86-28.46°C (mean of 20.10°C)
Wind speed:	0-5.835 m/s
Relative humidity:	29.83-100.9% (mean of 73.5%)

C. Test Design

Guideline Criteria	Reported Information
Range finding test?	No
Reference toxicant tested?	Yes, diflubenzuron 480 g/L SC.
Application Rate	3.3 mL/L (maximum commercially recommended rate)

Guideline Criteria	Reported Information
Method of administration:	• The 50% (w/v) sucrose solution was prepared with reverse osmosis-treated water. • The test substance,"RIMON" 10EC, was dispersed in the 50% sucrose solution to obtain 3.3 mL of product/L. The toxic reference product, diflubenzuron 480 g/L SC, was dispersed in the 50% sucrose solution to obtain 1.2 mL of product/L . • The one liter doses were administered using commercial beehive feeders and were consumed within 63 hours. • The 50% sucrose solutions for the treatment group and toxic reference were not analyzed.
Sufficient number of time periods to yield statistically sound data?	Colonies were observed between July 22, 1997-August 12, 1997.
Controls: Negative control and/or diluent/solvent control	Negative (50% w/v sucrose solution) control
Number of colonies per group:	Four replicate hive colonies (approximately 10,000-15,000 bees each) per treatment and control group.
Solvent: Distilled water or the following solvents: acetone, dimethylformamide, triethylene glycol, methanol, ethanol.	N/A

Guideline Criteria	Reported Information
Observations and frequency:	• Brood development and behavior (e.g., flight intensity, worker bee, and queen behavior) were observed twice weekly. • Mortality in dead-bee traps were observed daily.

12. REPORTED RESULTS:

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Control performance:	Mortality was low in all of the sucrose control-treated hives, being less than 15% at the end of the study. The toxic reference product, diflubenzuron 480 g/L SC did not demonstrate significant ($p>0.05$) toxic effects on adult honeybees; however, substantial and unequivocal effects on brood development were observed.
Raw data included?	Yes
Signs of toxicity (if any) were described?	Developmental and behavior anomalies of exposed honeybee colonies were recorded with the date of observations.

Mortality

Group (mL/L "RIMON" 10EC) Nominal Concentration	Number of dead bees				
	Worker	Drone	Pupae	Larvae	Total
Control (sucrose)	748	58	59	0	865
3.3	417	66	108	1	592
Reference (diflubenzuron 480 g/L SC), 1.2 mL/L	486	65	44	0	595

Eggs (% Successful development of 100 bees selected at test initiation)

Group (mL/L "RIMON" 10EC) Nominal Concentration	Days After Treatment					
	2	7	10	14	16	21
Control (sucrose)	98	62	56	42	40	40
3.3	96	6*	2*	0*	0*	0*
Reference (diflubenzuron 480 g/L SC), 1.2 mL/L	88	21	1	1	0	0

* Significant difference compared to the sucrose control ($p<0.05$).

Young Larvae (% Successful development of 100 bees selected at test initiation)

Group (mL/L "RIMON" 10EC) Nominal Concentration	Days After Treatment					
	2	7	10	14	16	21
Control (sucrose)	94	69	65	56	55	55

Group (mL/L "RIMON" 10EC) Nominal Concentration	Days After Treatment					
3.3	73	6*	3*	2*	2*	2*
Reference (diflubenzuron 480 g/L SC), 1.2 mL/L	68	17	7	6	6	6

* Significant difference compared to the sucrose control ($p<0.05$).

Old Larvae (% Successful development of 100 bees selected at test initiation)

Group (mL/L "RIMON" 10EC) Nominal Concentration	Days After Treatment					
	2	7	10	14	16	21
Control (sucrose)	97	84	78	78	78	78
3.3	93	29*	21*	21*	21*	21*
Reference (diflubenzuron 480 g/L SC), 1.2 mL/L	99	52	37	37	37	35

* Significant difference compared to the sucrose control ($p<0.05$).

Reported Results:

The percentage of eggs that failed to develop successfully were 49.52, 100, and 99.74% in the control, "RIMON" EC, and diflubenzuron treatment groups, respectively. The percentage of young larvae that failed to develop successfully were 39.23, 97.75, and 93.95% in the control, "RIMON" EC, and diflubenzuron treatment groups, respectively. The percentage of old larvae that failed to develop successfully were 18.75, 78.53, and 64.36% in the control, "RIMON" EC, and diflubenzuron treatment groups, respectively.

The mean number of dead worker bees from each hive per assessment were 8.5, 4.7, and 5.5 in the control, "RIMON" EC, and diflubenzuron treatment groups, respectively. The mean number of dead drone bees from each hive per assessment were 0.66, 0.75, and 0.74 in the control, "RIMON" EC, and diflubenzuron treatment groups, respectively.

There were significant effects ($p<0.05$) on brood development (eggs, young larvae, and old larvae) in the 3.3 mL"RIMON" 10EC/L treatment group compared to the control. There was no significant difference in mortality of the sucrose control and "RIMON" 10EC treatment group. No other treatment related effects were observed during the study. The LC_{50} could not be determined, but it was presumed to be >3.3 mL"RIMON" 10EC/L.

Statistical Method: The adult and brood mortalities were analyzed using the Kruskal-Wallis test to compare the number of dead bees (at worker, drone, pupal, and larval stages). The brood development was analyzed using the Kruskal-Wallis test to compare the number of successfully developed eggs, young larvae, and old larvae out of 100 selected brood cells. Stages were analyzed separately for each time period. In cases where all groups were significantly different, a further comparison was made between the control and groups treated with "RIMON" 10EC. The statistical analyses were performed using SAS 6.11 (1989, 1996).

13. VERIFICATION OF STATISTICAL RESULTS:

The sucrose control group was compared to the "RIMON" 10EC group for all developmental stages at all time periods using a Student's t-test. Mortality was also compared for all developmental stages (i.e., workers, drones, pupae, and larvae).

The reviewer's analysis detected significant differences in brood development at all stages. No significant differences were detected for mortality.

14. REVIEWER'S COMMENTS:

The reviewer's conclusions agreed with the study author's. There were significant adverse effects of "RIMON" 10EC on brood development at the egg, young and old larval stages. Adverse effects appeared at 2 (young larvae) and 7 (eggs and old larvae) days after treatment and persisted over the 21-day study. There were no effects of treatment on bee mortality.

The insecticide tested, "RIMON" 10EC, has a primary mode of action as a chitin synthesis inhibitor.

The study author reported development as "successful", "unsuccessful", and "unreliable". Only successful development was analyzed. The normal order for successful brood development was egg to young larvae to old larvae to capped to uncapped. The exceptions to this successful development order included: (1) if one stage of development was skipped from the previous assessment (if two stages were skipped between 5-day assessment periods), (2) if a cell was capped for less than 3 continuous assessments and not followed by a dead pupae, (3) if no more than two successive observations of either eggs, young larvae, or old larvae were made.

This study is scientifically sound; however, the study is classified as Supplemental because there is not an EPA-approved protocol for this type of study. The information that it provides may be useful for risk assessment purposes.

15. REFERENCES:

Barrett, K.L., Grandy, N., Harrison, E.G., Hassan, S., and Oomen, P. (1995). *Guidance document on regulatory testing procedures for pesticides with non-target arthropods*. From the Workshop European standard Characteristics of Beneficials Regulatory Testing (ESCORT). SETAC-Europe (1995), pp. 51, ISBN 0 9522535 2 6.

OEPP/EPPO (1992) Guideline on test methods for evaluating the side-effects of plant protection products, No. 170. Honeybees. *Bulletin* OEPP/EPPO *Bulletin* 22, 203-216.

OEPP/EPPO (1993) Decision-making scheme for the environmental risk assessment of plant protection products. *Bulletin* OEPP/EPPO *Bulletin* 23, 151-165.

Oomen, P.A., De Ruijter, A. & Van Der Steen, J. (1992) Method for honeybee brood feeding tests with insect growth-regulating insecticides. *Bulletin* OEPP/EPPO *Bulletin* 22, 613-616.

SAS Institute (1989) *SAS/STAT User's Guide, Version 6, Fourth Edition, Vol.*2. SAS Institute Inc., Cary, NC, USA.

SAS Institute (1996) *SAS/STAT Software: Changes and Enhancements through Release 6.11.* SAS Institute, Cary, NC, USA.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL RESULTS:

Eggs Day 2

t-Test: Two-Sample Assuming Equal Variances

	Variable 1	*Variable 2*
Mean	98	96
Variance	4	24.66667
Observations	4	4
Pooled Variance	14.33333	
Hypothesized Mean Difference	0	
df	6	
t Stat	0.747087	
P(T<=t) one-tail	0.241625	
t Critical one-tail	1.943181	
P(T<=t) two-tail	0.483249	
t Critical two-tail	2.446914	

Young Larvae Day 2

t-Test: Two-Sample Assuming Equal Variances

	Variable 1	*Variable 2*
Mean	94	73
Variance	90.66667	311.3333
Observations	4	4
Pooled Variance	201	
Hypothesized Mean Difference	0	
df	6	
t Stat	2.09477	
P(T<=t) one-tail	0.040531	
t Critical one-tail	1.943181	
P(T<=t) two-tail	0.081062	
t Critical two-tail	2.446914	

Old Larvae day 2

	Variable 1	*Variable 2*
Mean	97.25	92.75
Variance	8.916667	141.5833
Observations	4	4
Pooled Variance	75.25	
Hypothesized Mean Difference	0	
df	6	
t Stat	0.733625	
P(T<=t) one-tail	0.245419	
t Critical one-tail	1.943181	

P(T<=t) two-tail	0.490838
t Critical two-tail	2.446914

Eggs Day 7

t-Test: Two-Sample Assuming Equal Variances

	Variable 1	*Variable 2*
Mean	62.25	6.25
Variance	473.5833	80.25
Observations	4	4
Pooled Variance	276.9167	
Hypothesized Mean Difference	0	
df	6	
t Stat	4.75914	
P(T<=t) one-tail	0.001565	
t Critical one-tail	1.943181	
P(T<=t) two-tail	0.003129	
t Critical two-tail	2.446914	

Young Larvae Day 7

t-Test: Two-Sample Assuming Equal Variances

	Variable 1	*Variable 2*
Mean	69.25	5.75
Variance	402.25	79.58333
Observations	4	4
Pooled Variance	240.9167	
Hypothesized Mean Difference	0	
df	6	
t Stat	5.785692	
P(T<=t) one-tail	0.000583	
t Critical one-tail	1.943181	
P(T<=t) two-tail	0.001166	
t Critical two-tail	2.446914	

Old Larvae Day 7

t-Test: Two-Sample Assuming Equal Variances

	Variable 1	*Variable 2*
Mean	84.25	28.75
Variance	120.9167	491.5833
Observations	4	4
Pooled Variance	306.25	
Hypothesized Mean Difference	0	
df	6	
t Stat	4.485077	
P(T<=t) one-tail	0.002085	

t Critical one-tail	1.943181
P(T<=t) two-tail	0.00417
t Critical two-tail	2.446914

Total Dead Workers

t-Test: Two-Sample Assuming Equal Variances

	Variable 1	*Variable 2*
Mean	187	104.25
Variance	5718.667	3104.917
Observations	4	4
Pooled Variance	4411.792	
Hypothesized Mean Difference	0	
df	6	
t Stat	1.761877	
P(T<=t) one-tail	0.06428	
t Critical one-tail	1.943181	
P(T<=t) two-tail	0.128561	
t Critical two-tail	2.446914	

Total Dead Drones

t-Test: Two-Sample Assuming Equal Variances

	Variable 1	*Variable 2*
Mean	14.5	16.5
Variance	86.33333	737.6667
Observations	4	4
Pooled Variance	412	
Hypothesized Mean Difference	0	
df	6	
t Stat	0.13935	
P(T<=t) one-tail	0.446868	
t Critical one-tail	1.943181	
P(T<=t) two-tail	0.893736	
t Critical two-tail	2.446914	

Total Dead Pupae

t-Test: Two-Sample Assuming Equal Variances

	Variable 1	*Variable 2*
Mean	14.75	27
Variance	444.9167	820.6667
Observations	4	4
Pooled Variance	632.7917	

Hypothesized Mean Difference	0
df	6
t Stat	-0.68869
P(T<=t) one-tail	0.258376
t Critical one-tail	1.943181
P(T<=t) two-tail	0.516752
t Critical two-tail	2.446914

Total Dead Larvae

t-Test: Two-Sample Assuming Equal Variances

	Variable 1	*Variable 2*
Mean	0	0.25
Variance	0	0.25
Observations	4	4
Pooled Variance	0.125	
Hypothesized Mean Difference	0	
df	6	
t Stat	-1	
P(T<=t) one-tail	0.177959	
t Critical one-tail	1.943181	
P(T<=t) two-tail	0.355918	
t Critical two-tail	2.446914	